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(54) METHOD OF TESTING ANTICANCER AGENT-SENSITIVITY OF TUMOR CELLS

(57) Susceptibility of a tumor cell to an anticancer agent is tested by measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in a tumor cell collected from a cancer patient treated with an anticancer agent (E7070 and related compounds thereof), or causing the anticancer agent to act on a tumor cell collected from a cancer patient and measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in the tumor cell, and determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease.

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Description

Technical Field

[0001] The present invention relates to a method for testing whether a tumor cell is susceptible to an anticancer agent or not.

Background Art

[0002] In a conventional clinical study of an anticancer agent, its toxicity profile and maximum recommended dose are first determined in the Phase I clinical study, and then the agent is evaluated as a drug based on the response rate using the tumor reduction rate as a criterion for efficacy in the Phase II clinical study. Meanwhile, with the advancement in cancer biology in recent years, drugs having a novel action mechanism for inhibiting the intracellular signal transduction system, angiogenesis and so forth are in the course of active research and development. For these novel anticancer agents, it may be possible that a maximum recommended dose close to a toxic dose does not necessarily need to be administered. Further, it is estimated that drug efficacy could be more appropriately judged by using improvement of QOL (Quality of Life) or prolongation of life associated with tumor growth inhibition as an index rather than tumor reduction. In this case, to more logically and specifically confirm the drug efficacy, it is desirable to utilize change of a biological marker closely relating to a tumor growth inhibition mechanism as a surrogate marker.

[0003] In the anticancer therapy, in general, reactivity of a living body when an anticancer agent is administered is largely depends on susceptibility of a tumor cell, which is a target of the drug, to the drug. Generally, this susceptibility of tumor cell to the drug greatly varies in every type of tumor cell. Such differences in susceptibility are attributable to quantitative or qualitative differences of target molecules of the drug or factors relating to the molecules, acquisition of drug resistance and so forth. Considering such a background, it would be very useful if change in a tumor cell specifically caused when the tumor cell as a target exhibits susceptibility to a drug can be measured by using tumor tissue obtained by biopsy etc., because early determination of drug efficacy, establishment of a treatment method, selection of a new treatment method and so forth become possible by using the change as a surrogate marker. Further, if a tumor cell is isolated from tumor tissue obtained by biopsy or the like in a conventional manner prior to a treatment and then treated with a drug, and whether this tumor cell is susceptible to the drug is determined based on change of the aforementioned surrogate marker, it becomes possible to preliminarily predict whether the treatment using the drug is effective or not, and this would be extremely useful in clinical practice. It is important that the change of this surrogate marker should be specific to antitumor effect, and it is sufficient that the change can be measured with high sensitivity. Specifically, quantification of variations in the gene expression specific to the antitumor effect of the drug, analysis of quantitative variations of a protein along with the changes in gene expression, analysis of functional changes associated with these changes and so forth can be used as the surrogate markers.

[0004] E7070 (N-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide) is a compound that has an antitumor effect targeting the G1 phase of the cell cycle, and is being clinically developed (Takashi Owa, Hiroshi Yoshino, Tatsuo Okauchi, Kentaro Yoshimatsu, Yoichi Ozawa, Naoko Hata Sugi, Takeshi Nagasu, Nozomu Koyanagi and Kyosuke Kitoh, J. Med. Chem, 1999, 42, 3789-3799).

[0005] Spectra of intensity of the growth inhibitory action of this compound on various tumor cells are different from those of any of existing anticancer agents, and this compound is expected to have an effect as an anticancer agent having a novel action mechanism. In order to accelerate the clinical development of this drug and early establish a clinical treatment method, and contribute to improvement of QOL of patients by efficiently advancing treatment based on established treatment methods, it is desirable to discover and apply a surrogate marker that can be specifically used upon the administration of the drug.

[0006] In recent years, methods of using various DNA microarrays to detect expression levels of a large number of genes at the same time have been established and widely used (Schena M, Shalon D, Davis RW, Brown PO, Science 1995, 270, 467-70; Lockhart, D.J., Dong, H., Byrne, M.C., Follettie, M.T., Gallo, M.V., Chee, M.S., Mittmann, M., Wang C., Kobayashi, M., Horton, H. Brown, E.L., Nature Biotechnology, 1996, 14, 1675-1680).

[0007] Also in the field of cancer studies, researches using such DNA microarrays are actively being conducted. For example, in a study in which diffuse large B-cell lymphoma (DLBCL) was investigated by expression analysis using a DNA microarray, DLBCL has been classified into two of different types according to differences in gene expression profiles, and it has been shown that this classification leads to prediction of prognosis (Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Staudt LM, et al, Nature, 2000, 403, 503-11). Further, there have been a report in which, by analyzing gene expression profiles of a panel of 60 types of cancer cell lines from the National Cancer Institute in the United States, these cell lines were reclassified and their characteristics were examined (Ross DT, Scherf U, Eisen MB, Perou CM, Rees C,

Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M, Pergamenschikov A, Lee JC, Lashkari D, Shalon D, Myers TG, Weinstein JN, Botstein D, Brown PO, Nat Genet, 2000, 24, 227-35), a report in which relationships of the gene expression profiles of the panel of 60 types of cancer cell lines and susceptibility to various anticancer agents of each cell line were discussed (Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, Scudiero DA, Eisen MB, Sausville EA, Pommier Y, Botstein D, Brown PO, Weinstein JN, Nat Genet, 2000, 24, 236-44) and so forth.

[0008] Further, there have also been several reports in which changes in gene expression that occurred when anticancer agents were caused to act on tumor cells were examined by similarly using a DNA microarray (partly macroarray using a membrane filter) (Rhee CH, Ruan S, Chen S, Chenchik A, Levin VA, Yung AW, Fuller GN, Zhang W, Oncol Rep, 1999, 6, 393-401. Zimmermann J, Erdmann D, Lalande I, Grossenbacher R, Noorani M, Furst P, Oncogene, 2000, 19, 2913-20. Kudoh K, Ramanna M, Ravatn R, Elkahloun AG, Bittner ML, Meltzer PS, Trent JM, Dalton WS, Chin KV, Cancer Res, 2000, 4161-6). These reports show that the analysis of variations in gene expression is extremely useful in comparison of characteristics of two or more cell populations and comprehensive studies of biological changes of cells caused by drug treatment or the like at a molecular level.

Disclosure of the Invention

[0009] An object of the present invention is to provide surrogate markers for antitumor effect of E7070 and its related compounds in cases that the compounds are caused to act on tumor cells.

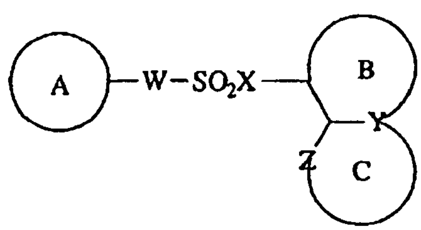
[0010] The inventors of the present invention analyzed changes in gene expression caused when E7070 and its related compounds were caused to act on tumor cells susceptible to these anticancer agents by the DNA microarray method and found changes in gene expression caused by these anticancer agents in common.

[0011] Further, they also found that, among these genes, there were genes that exhibited changes of expression common to three kinds of cancers and that expression changes of these genes can be used as surrogate markers for the antitumor effect of E7070 and its related compounds, and thus accomplished the present invention.

[0012] The present invention provides the followings.

1. A method for testing susceptibility of a tumor cell to an anticancer agent, which comprises:

   1) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in a tumor cell collected from a cancer patient treated with an anticancer agent represented by the following general formula (I):

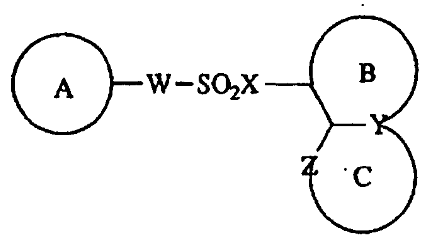


(I)

   wherein A represents a monocyclic or bicyclic aromatic ring, which may be substituted,
   B represents a 6-membered unsaturated hydrocarbon ring or a 6-membered unsaturated heterocyclic ring containing one nitrogen atom as a heteroatom, each of which may be substituted,
   C represents a 5-membered heterocyclic ring containing one or two nitrogen atoms, which may be substituted,
   W represents a single bond or -CH=CH-,
   X represents -N(R^1)- or an oxygen atom,
   Y represents a carbon or nitrogen atom,
   Z represents -N(R^2)- or a nitrogen atom, and
   R^1 and R^2 may be identical or different and each represent a hydrogen atom or a lower alkyl group, and
   2) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in a tumor cell collected from the cancer patient before the treatment with the anticancer agent.

2. A method for testing susceptibility of a tumor cell to an anticancer agent, which comprises:

1) causing an anticancer agent to act on a tumor cell collected from a cancer patient, the said anticancer agent being represented by the following general formula (I):



(I)

wherein A represents a monocyclic or bicyclic aromatic ring, which may be substituted,
B represents a 6-membered unsaturated hydrocarbon ring or a 6-membered unsaturated heterocyclic ring containing one nitrogen atom as a heteroatom, each of which may be substituted,
C represents a 5-membered heterocyclic ring containing one or two nitrogen atoms, which may be substituted,
W represents a single bond or -CH=CH-,
X represents -N(R^1)- or an oxygen atom,
Y represents a carbon or nitrogen atom,
Z represents -N(R^2)- or a nitrogen atom, and
R^1 and R^2 may be identical or different and each represent a hydrogen atom or a lower alkyl group,
2) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in the tumor cell, and
3) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in an untreated tumor cell.

3. The method according to 1 or 2, wherein the expression level or levels of the gene or genes are measured by quantifying an RNA or RNAS which are a transcription product or products of the gene or genes by using a DNA microarray.
4. The method according to 1 or 2, wherein the expression level or levels of the gene or genes are measured by quantifying an RNA or RNAs which are a transcription product or products of the gene or genes by quantitative PCR.
5. A reagent for quantifying an RNA for use in the method as defined in 4, which comprises an oligonucleotide complementary to the RNA as a component.
6. The method according to 1 or 2, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product products of the gene or genes by an immunochemical method.
7. The method according to 6, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product or products of the gene or genes by ELISA.
8. The method according to 6, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product or products of the gene or genes by Western blotting.
9. An immunoassay reagent used in the method as defined in 6, which comprises an antibody directed to the protein as a component.
10. The method according to 1 or 2, wherein A represents benzene or pyridine which may be substituted, B represents benzene which may be substituted, C represents pyrrole which may be substituted, W represents a single bond, and X and Z both represent -NH-.

Brief Description of the Drawings

[0013]

Fig. 1 shows cell growth inhibition curves obtained with E7070, of cell strains HCT116-C9, HCT116-C9-C1, LX-1 and LX-1-E2.
Fig. 2 shows results of analysis of variations in gene expression in the E7070-susceptible strain HCT116-C9 by

quantitative PCR.

Fig. 3 shows results of analysis of variations in gene expression in the E7070-susceptible strain LX-1 by quantitative PCR.

Fig. 4 shows results of analysis of variations in gene expression in the E7070-resistant strain HCT116-C9-C1 by quantitative PCR.

Fig. 5 shows results of analysis of variations in gene expression in the E7070-resistant strain LX-1-E2 by quantitative PCR.

Best Mode for Carrying out the Invention

[0014] Hereafter, embodiments of the present invention will be described in detail.

[0015] The test method of the present invention is characterized in that a change in expression level of a gene in a tumor cell caused when the tumor cell is exposed to an anticancer agent *in vivo* or *in vitro* is used as an index of susceptibility of the tumor cell to the anticancer agent.

[0016] Therefore, the test method of the first embodiment of the present invention comprises the steps of 1) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 of a tumor cell collected from a cancer patient treated with an anticancer agent represented by the general formula (I), and 2) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in a tumor cell collected from the cancer patient before the treatment with the anticancer agent.

[0017] Further, the test method of the second embodiment of the present invention comprises the steps of 1) causing an anticancer agent represented by the general formula (I) to act on a tumor cell collected from a cancer patient,
2) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in the tumor cell, and
3) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in an untreated tumor cell.

[0018] The anticancer agent used in the present invention is a sulfonamide derivative or a sulfonnic acid ester derivative represented by the general formula (I).

[0019] In the general formula (I), the "monocyclic or bicyclic aromatic ring, which may be substituted" represented by A is an aromatic hydrocarbon ring or an aromatic heterocyclic ring containing at least one of nitrogen, oxygen and sulfur atoms, each of which may have one to three substituents thereon. Examples of such aromatic rings included in the ring A include pyrrole, pyrazole, imidazole, thiophene, furan, thiazole, oxazole, benzene, pyridine, pyrimidine, pyrazine, pyridazine, naphthalene, quinoline, isoquinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, indole, isoindole, indolizine, indazole, benzofuran, benzothiophene, benzoxazole, benzimidazole, benzopyrazole, benzothiazole and so forth. They may have one to three substituents. When two or more substituents are present, they may be the same or different. Examples of the substituents include an amino group which may be substituted with a lower alkyl or lower cycloalkyl group, a lower alkyl group, a lower alkoxy group, hydroxyl, nitro, mercapto, cyano, a lower alkylthio group, halogen, a group represented by the formula -a-b [wherein a represents a single bond, $-(CH_2)_k-$, $-O-(CH_2)_k-$, $-S-(CH_2)_k-$ or - $N(R^3)-(CH_2)_k-$ (k is an integer of 1 to 5, R^3 represents a hydrogen atom or a lower alkyl group), and b represents $-CH_2-$ d (wherein d represents an amino group which may be substituted with a lower alkyl group, halogen, hydroxyl, a lower alkylthio group, cyano or a lower alkoxy group)], a group represented by the formula -a-e-f [wherein a has the same meaning as defined above, e represents -S(O) or $-S(O)_2-$, f represents an amino group which may be substituted with a lower alkyl or lower alkoxy group, a lower alkyl group, trifluoromethyl, $-(CH_2)_m-b$ or $-N(R^4)-(CH_2)_m-b$ (wherein b has the same meaning as defined above, R^4 represents a hydrogen atom or a lower alkyl group, and m is an integer of 1 to 5)], a group represented by the formula -a-g-h [wherein a has the same meaning as defined above, g represents -C(O)- or -C(S)-, h represents an amino group which may be substituted with a lower alkyl group, hydroxyl, a lower alkyl group, a lower alkoxy group, $-(CH_2)_n-b$ or $-N(R^5)-(CH_2)_n-b$ (wherein b has the same meaning as defined above, R^5 represents a hydrogen atom or a lower alkyl group, and n is an integer of 1 to 5)], a group represented by the formula $-a-N(R^6)-g-i$ [wherein a and g have the same meanings as defined above, R^6 represents a hydrogen atom or a lower alkyl group, i represents a hydrogen atom or a lower alkoxy group or f (f has the same meaning as defined above)], a group represented by the formula $-a-N(R^7)-e-f$ (wherein a, e and f have the same meanings as defined above, and R^7 represents a hydrogen atom or a lower alkyl group) and group represented by the formula $-(CH_2)_p-j-(CH_2)_q-b$ (wherein j represents an oxygen or sulfur atom, b has the same meaning as defined above, and p and q may be the same or different and each represent an integer of 1 to 5) and so forth.

[0020] When the substituent is an amino group substituted with two of alkyl groups, both of the alkyl groups may bond to form a 5- or 6-membered ring. Further, when A is a nitrogen-containing heterocyclic ring having hydroxyl or

mercapto, these groups may present in the form of an oxo or thioxo group by resonance.

[0021] The "6-membered unsaturated hydrocarbon ring or 6-membered unsaturated heterocyclic ring containing one nitrogen atom as a heteroatom, which may be substituted" represented by B means benzene or pyridine which may be partially hydrogenated. It may have one or two of substituents on the ring, and when two of substituents are present, they may be the same or different.

[0022] The "5-membered heterocyclic ring containing one or two nitrogen atoms, which may be substituted" represented by C means pyrrole, pyrazole or imidazole which may be partially hydrogenated. It may have one or two of substituents on the ring, and when two of substituents are present, they may be the same or different.

[0023] Examples of the substituents that the rings of B and C may have include halogen, cyano, a lower alkyl group, a lower alkoxy group, hydroxyl, oxo, a group represented by the formula -C(O)-r (wherein r represents a hydrogen atom, an amino group which may be substituted with a lower alkyl group, a lower alkyl group, a lower alkoxy group or hydroxyl), an amino group substituted with a lower alkyl group, trifluoromethyl and so forth.

[0024] In the general formula (I), the lower alkyl group in the definitions of R^1 and R^2 as well as the substituents that the rings of A, B and C may have means a linear or branched alkyl group having 1 to 6 carbon atoms, and examples thereof include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl (amyl), isopentyl, neopentyl, tert-pentyl, 1-methylbutyl, 2-methylbutyl, 1,2-dimethylpropyl, n-hexyl, isohexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl and so forth. Among these, methyl, ethyl, n-propyl, isopropyl, n-butyl and isobutyl are preferred, and methyl, ethyl, n-propyl and isopropyl are most preferred.

[0025] The lower cycloalkyl group mentioned in the definitions of the substituents that the ring of A may have means a cycloalkyl group having 3 to 8 carbon atoms, and examples thereof include cyclopropyl, cyclopentyl, cyclohexyl and so forth.

[0026] The lower alkoxy mentioned in the definitions of the substituents that the rings of A, B and C may have means an alkoxyl group derived from the aforementioned lower alkyl group, such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy and tert-butoxy. Among these, methoxy and ethoxy are most preferred. The lower alkylthio group means an alkylthio group derived from the aforementioned lower alkyl group. Further, examples of the halogen include fluorine, chlorine, bromine and so forth.

[0027] The sulfonamide derivatives or sulfonic acid ester derivatives represented by the general formula (I) may form a salt with an acid or a base. The anticancer agent used in the present invention also includes salts of the compounds represented by the general formula (I). Examples of the salt with an acid include salts with inorganic acids, such as hydrochlorides, hydrobromides and sulfates, and salts with organic acids such as acetic acid, lactic acid, succinic acid, fumaric acid, maleic acid, citric acid, benzoic acid, methanesulfonic acid and p-toluenesulfonic acid, and examples of the salt with a base include salts with inorganic bases, such as sodium salts, potassium salts and calcium salts and salts with organic bases such as triethylamine, arginine and lysine.

[0028] It is needless to say that the compounds include hydrates and optical isomers of these compounds if they are present. The anticancer agent used in the present invention exhibits a high antitumor activity, and the anticancer agents also includes compounds which undergo metabolism such as oxidation, reduction, hydrolysis and conjugation *in vivo* to exhibit the antitumor activity. Further, the anticancer agent used in the present invention includes compounds which undergo metabolism *in vivo* such as oxidation, reduction and hydrolysis to form compounds represented by the general formula (I).

[0029] Tumor cells of which susceptibility is tested are not particularly limited so long as they are susceptible to the anticancer agent represented by the general formula (I). Examples thereof include tumor cells derived from colon cancer, lung cancer, breast cancer, leukemia, pancreas cancer, kidney cancer, melanoma, malignant lymphoma, head and neck cancer, gastric cancer and so forth.

[0030] The tumor cells collected from a cancer patient include tumor cells contained in a cancer tissue isolated from the cancer patient.

[0031] In the test method of the first embodiment, the tumor cell collected from a cancer patient may be a tumor cell collected from a cancer patient treated with an anticancer agent represented by the general formula (I) in such an amount that susceptibility of the tumor cell can be measured. Usually, tumor cells collected from a cancer patient treated with a dose of 100 to 1500 mg for 1 to 14 days are used.

[0032] In the test method of the second embodiment, conditions for causing the anticancer agent represented by the general formula (I) to act on a tumor cell collected from a cancer patient are not limited so long as susceptibility of the tumor cells can be measured, and culture is usually performed in a medium containing the anticancer agent at a concentration of 0.01 to 10 μM for 6 to 72 hours.

[0033] The gene expression level can be measured by quantifying RNA as a gene transcription product or a protein as a gene product. The RNA or protein can be quantified usually by extracting the RNA or protein from tumor cells and quantifying the RNA or protein in the extract. Hereafter, examples of 1. extraction of RNA or protein, 2. quantification

of RNA, and 3. quantification of protein will be explained in detail in this order.

1. Extraction of RNA

1) Extraction of RNA or protein from cancer tissue of patient treated with anticancer agent represented by general formula (I)

[0034] RNA or protein is extracted from a cancer tissue collected by biopsy or the like from a patient treated with an anticancer agent represented by the general formula (I) by the method described below.

[0035] RNA can be extracted according to a usual RNA extraction method. For example, extraction can be carried out by using TRIZOL Reagent (Life Technologies Oriental) or the like according to the attached operation manual. Specifically, a cancer tissue with 1 ml of TRIZOL reagent per 50 to 100 mg of the cancer tissue is homogenized by using a Teflon homogenizer. The mixture is centrifuged (12,000 x g, 10 minutes, 4°C), and the obtained supernatant is left at room temperature for 5 minutes and 0.2 ml of chloroform is added thereto per 1 ml of the used TRIZOL Reagent. The resulting solution is vigorously stirred by shaking for 15 seconds, left at room temperature for 2 to 3 minutes and then centrifuged (12,000 x g, 15 minutes, 4°C). After the centrifugation, the aqueous layer is transferred to a new tube, and 0.5 ml of isopropyl alcohol is added per 1 ml of the used TRIZOL reagent. The mixture is left at room temperature for 10 minutes and centrifuged (12,000 x g, 10 minutes, 4°C). The obtained precipitates are washed with 75% ethanol, air-died and used in subsequent operations as total RNA.

[0036] Protein can be extracted from a cancer tissue according to the methods described in Bollag, D.M., Rozycki M.D., Edelstein S.J., Protein Methods, 1996, Wiley-Liss, Inc., New York, U.S.A.; Walker, J.M., The protein handbook, 1996, Humana Press, New Jersey, U.S.A. and so forth.

2) Extraction of RNA or protein from cancer cell cultured in the presence of anticancer agent represented by general formula (I)

[0037] Cancer cells (tumor cells) are isolated in a conventional manner from a cancer tissue obtained from a patient by biopsy or the like. For example, according to the method of Hamburger et al. (Hamburger A., Salmon S.E., Kim M. B., Trent J.M., Soehnlen B.J., Alberts D.S. and Schmidt H.T., Cancer Res., 38, 3438-3443, 1978), the obtained tissue is aseptically chopped, and then a cell suspension is prepared by using a stainless mesh, injection needle, nylon mesh or the like. The obtained cells are cultured in a suitable medium (for example, RPMI-1640, MEM, McCoy medium etc. containing 10-15% FCS). The obtained cancer cells are cultured at 37°C in the presence of the anticancer agent represented by the general formula (I) under 5% CO_2 condition for a suitable period, preferably, for 3, 6, 12 or 24 hours, more preferably for 12 hours, and RNA or protein is extracted by the method described below. Further, it is also possible to specifically isolate only cancer cells from a tissue obtained by biopsy or the like by using the soft agar culture method (Hamburger A., and Salmon S. E., Science, 197, 461-463, 1977; Hamburger A., and Salmon S. E., J. Clin. Invest., 60, 846-854, 1977; Von Hoff D. D., and Johnson, G. E., Proc. Am. Assoc. Cancer Res., 20, 51, 1979) and use them.

[0038] RNA can be extracted from a cancer cell according to a usual RNA extraction method as the extraction of RNA from a cancer tissue. For example, when TRIZOL Reagent (Life Technologies Oriental) is used, extraction can be carried out according to the attached operation manual. Specifically, 1 ml of TRIZOL Reagent is added per 5 to 10 x 10^6 cancer cells, and the same operations as in the extraction of RNA from a cancer tissue can be performed.

[0039] Extraction of protein from cancer cells can also be carried out according to methods described in publications as in the extraction from cancer tissue.

2. Quantification of RNA

[0040] RNA can be quantified by techniques of Northern blotting analysis, DNA microarray analysis, RT-PCR, quantitative PCR and so forth. It is preferable to use a DNA microarray or quantitative PCR. These techniques will be explained below, but the present invention is not limited to them.

[0041] Quantification by using a DNA microarray is performed as follows. First, double-stranded cDNA is synthesized by using the obtained RNA as a template, Superscript Choice System (Life Technologies Oriental) and T7-d(T)$_{24}$ primer. Subsequently, biotinylated cRNA is synthesized by using the cDNA as a template.

[0042] Specifically, single-stranded DNA is first synthesized from the obtained RNA by using the T7-d(T)$_{24}$ primer. Then, dNTP, DNA ligase, DNA polymerase I and RNase H are added thereto to react them and T4 DNA polymerase I is further added thereto to complete the synthesis of double-stranded cDNA. The obtained cDNA is purified, and then biotinylated UTP and CTP are added to allow a labeling reaction by using RNA Transcript Labeling Kit (Enzo Diagnostics). The reaction product is purified and heated at 94°C in 200 mM Tris/acetate (pH 8.1), 150 mM magnesium acetate, 50 mM potassium acetate for 35 minutes to obtain fragmented cRNA.

[0043] The fragmented cRNA is hybridized with GeneChip Hu6800 (Affymetrix) or an update product, for example, in 100 mM MES, 1 M sodium salt, 20 mM EDTA, 0.01% Tween 20 at 45°C for 16 hours. After the hybridization, the GeneChip is washed and stained according to the protocol EukGE-WS2 attached to the Affymetrix Fluidics Station. For the staining, streptavidin-phycoerythrin and biotinylated goat anti-streptavidin antibody are used. The stained GeneChip is scanned by using HP Argon Ion Laser Confocal Microscope (Hewlett Packard) to measure its fluorescence intensity. When this fluorochrome is used, fluorescence is measured at 570 nm by using an excitation light at 488 nm.

[0044] The quantitative data analysis is preferably conducted by using GeneChip Software (Affymetrix). To quantify RNA, an average of "differences [(Perfect match hybridization signal) - (Mismatch signal)]" is obtained for each probe family. When this value is 50 or higher, and quantitative values of RNA obtained under two kinds of conditions diverge, preferably when they diverge 1.8 times or more, it is determined that the gene expression has significantly "increased" or "decreased".

[0045] When the RNA level or levels of the gene or genes listed in Table 3 increase, or the RNA level or levels of the gene or genes listed in Table 4 decrease, it is determined that the tumor cells are susceptible to the anticancer agent.

[0046] Further, quantitative PCR is performed as follows by using SYBR Green and ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems).

[0047] The operation is performed in two steps of a reverse transcription reaction and PCR reaction. The reverse transcription reaction as the first step is carried out by adding dNTP, oligo $d(T)_{16}$ primer, RNase inhibitor mixture and Multiscribe Reverse Transcriptase (Perkin-Elmer Applied Biosystems) to the obtained RNA, keeping the mixture at 25°C for 10 minutes and then heating the mixture at 48°C for 30 minutes. The reaction is terminated by heating at 95°C for 5 minutes.

[0048] The obtained cDNA is subjected to the PCR reaction as the second step. The PCR reaction is carried out, for example, in a reaction system composed of 4 ng of cDNA, 1 x SYBR PCR buffer, 3 mM $MgCl_2$, 200 μM each of dATP, dCTP and dGTP, 400 μM dUTP, 200 nM primer pair, 0.01 U/μl AmpErase UNG and 0.025 U/μl AmpliTaq Gold DNA Polymerase (Perkin-Elmer Applied Biosystems). The PCR reaction is performed with, for example, reactions at 50°C for 2 minutes and at 95°C for 10 minutes followed by a cycle of reactions at 95°C for 20 seconds, at 55°C for 20 seconds and at 72°C for 30 seconds, which are repeated 40 times. The primers and the probe are designed, for example, by using Primer Expression (Perkin-Elmer Applied Biosystems). For comparison of two or more specimens, a quantified value is corrected into transcription amount in each specimen by using mRNA level of a house keeping gene, which shows little variation in transcription amount, preferably mRNA level of GAPDH, and used.

[0049] When the RNA level or levels of the gene or genes listed in Table 3 increase, or the RNA level or levels of the gene or genes listed in Table 4 decrease, it is determined that the tumor cells are susceptible to the anticancer agent.

[0050] The present invention also provides a reagent for quantifying RNA for use in the test method of the present invention, which comprises an oligonucleotide complementary to RNA as a transcription product of a gene of which expression level is to be measured, as a component. The oligonucleotide included as a component is a primer and/or probe used in quantitative PCR and can be designed as described above. The RNA-quantifying reagent of the present invention may contain components conventionally used in an ordinary quantifying reagent in addition to the above oligonucleotide.

3. Quantification of protein

[0051] Although protein is quantified based on its activity or antigenicity, quantification based on antigenicity, i.e., immunochemical quantification, which can be easily and generally applied to proteins, is preferred.

[0052] As for antibody directed to a protein, an antigen determinant is predicted from the amino acid sequence based on the report of Parker et al. (Parker J. M. R., Guo D., Hodges R. S., Biochemistry, 25,5425, 1986) or the report of Karplus et al. (Karplus P. A., Schulz G. E., Naturwissenschaften, 72, 212, 1985) to synthesize a peptide. Alternatively, a fusion protein, for example, a fusion protein with glutathione synthetase (GST) is expressed and purified by using a glutathione column to produce an antigen, and a rabbit, a mouse or the like is immunized with the obtained antigen to produce polyclonal or preferably monoclonal antibodies (Harlow E., Lane D., Antibodies: A Laboratory Manual, 1988, Cold Spring Harbor Laboratory Press, New York). Further, when commercially available antibodies are available, they can also be used after their specificity is confirmed.

[0053] By using the obtained antibodies, ELISA or RIA is performed to quantify the protein, for example, by the enzyme immunoassay method (Ishikawa E. et al., Igakushoin, 1982) or the method described in Ishikawa E., Kawai T., Miyai, K., Enzyme Immunoassay, Igakushoin, Tokyo, New York, 1981. When the protein is secreted out of the tumor cell, the protein in a medium can be quantified without extracting the protein from the tumor cell.

[0054] When the protein level or levels of the gene or genes listed in Table 3 increase, or the protein level or levels of the gene or genes listed in Table 4 decrease, it is determined that the tumor cells are susceptible to the anticancer agent.

[0055] The present invention also provides an immunoassay reagent for use in the test method of the present in-

vention, which comprises an antibody directed to the protein as a component. The antibody used as a component can be obtained as described above. The immunoassay reagent of the present invention may contain components conventionally used in ordinary immunoassay reagents in addition to the aforementioned antibody.

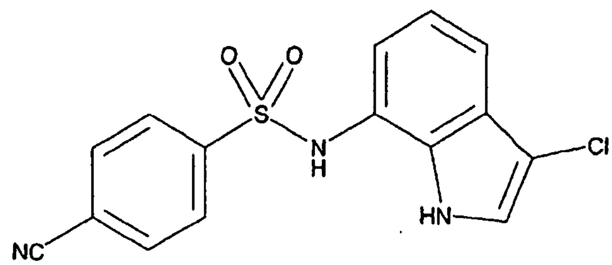
Examples

[0056] Hereafter, the present invention will be described more specifically with reference to the following specific examples. However, the present invention is not limited to them.

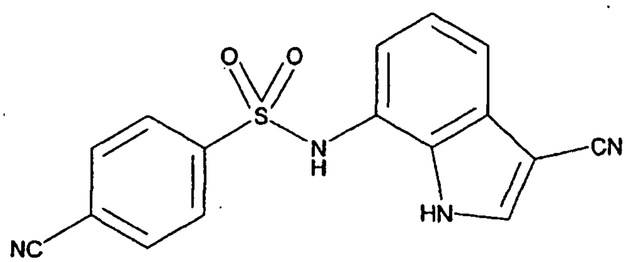
Example 1 Culture of E7070-susceptible and E7070-resistant strains and extraction of RNA

[0057] All cells were cultured in RPMI-1640 medium supplemented with 10% of fetal bovine serum, 100 units/ml of penicillin and 100 mg/ml of streptomycin at 37°C under 5% CO_2.

[0058] E7070, or ER-35748 or ER-68487 represented by the structural formula shown below was added to the media of an E7070-susceptible strain HCT116-C9 and an E7070-resistant strain HCT116-C9-C1 at a concentration of 8 μM and the cells were cultured. Then, cells were recovered after 0, 3, 6 and 12 hours. Further, the cells were also cultured for 12 hours without adding the drug and recovered.



ER-35748



ER-68487

[0059] Total RNA was extracted from these cells and subjected to the subsequent analyses. The RNA extracted from the cells cultured for 12 hours with the drug and the RNA from extracted the cells cultured for 12 hours without the drug were used for the gene expression analysis using a DNA microarray described in Example 2. HCT116-C9 was a substrain isolated from HCT116 derived from human colon cancer (American Type Culture Collection, Manassas, VA, U.S.A.), and HCT116-C9-C1 was an E7070-resistant substrain obtained by culturing this HCT116-C9 in the presence of E7070 with gradually increasing the E7070 concentration.

[0060] Similarly, E7070 was added to the media of an E7070-susceptible strain LX-1 and an E7070-resistant strain LX-1-E2 at a concentration of 8 μM and the cells were cultured. Cells were recovered after 0, 3, 6 and 12 hours. Total RNA was extracted from these cells and subjected to the subsequent analyses. LX-1 (Cancer Chemotherapy Center, Japan Foundation for Cancer Research, Tokyo, Japan) was a cell strain derived from human parvicellular lung cancer, and LX-1-E2 is an E7070-resistant substrain obtained by culturing this LX-1 in the presence of E7070 with gradually

increasing the E7070 concentration.

[0061] The used cell strains HCT116-C9, HCT116-C9-C1, LX-1 and LX-1-E2 were cultured for 72 hours with addition of E7070. Cell growth inhibition curves measured by the MTT method (Mosmann T., J. Immunol. Methods, 65, 55 (1983)) are shown in Fig. 1. The actual operation was performed by using CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) according to the attached operation manual.

[0062] Total RNA was extracted from the recovered cells by using TRIZOL Reagent (Life Technologies Oriental) according to the attached operation manual.

Example 2 Gene expression analysis using DNA microarray

1) Synthesis of cDNA and labeling with biotin

[0063] Each RNA obtained in Example 1 was dissolved in 100 μl of sterilized water treated with diethyl pyrocarbonate (DEPC) and further purified by using RNeasy Column (QIAGEN), and double-stranded cDNA was synthesized by using Superscript Choice System (Life Technologies Oriental) and a T7-d(T)$_{24}$ primer.

[0064] First, to 10 μg of RNA, 5 μM T7-d(T)$_{24}$ primer, 1 x First strand buffer, 10 mM DTT, 500 μM dNTP mix and 20 units/μl of Superscript II Reverse Transcriptase were added and the mixture was allowed to react at 42°C for 1 hour to synthesize single-stranded DNA. Subsequently, to the DNA, 1 x Second strand buffer, 200 μM dNTP mix, 67 U/ml of DNA ligase, 270 U/ml of DNA polymerase I and 13 U/ml of RNase H were added and the mixture was allowed to react at 16°C for 2 hours to synthesize double-stranded cDNA. Further, to the cDNA, 67 U/ml of T4 DNA polymerase I was added and the mixture was allowed to react at 16°C for 5 minutes and then 10 μl of 0.5 M EDTA was added to terminate the reaction.

[0065] The obtained cDNA was purified with phenol/chloroform, and a labeling reaction with biotinylated UTP and CTP was carried out by using RNA Transcript Labeling Kit (Enzo Diagnostics) according to the attached operation manual. The reaction product was purified with Rneasy Column and heated in 200 mM Tris/acetate buffer (pH 8.1), 150 mM magnesium acetate, 50 mM potassium acetate at 94°C for 35 minutes to obtain fragment cRNA.

2) Hybridization with DNA microarray (GeneChip) and measurement

[0066] The fragmented cRNA was hybridized with GeneChip Hu6800 (Affymetrix) in 100 mM MES, 1 M sodium salt, 20 mM EDTA and 0.01% Tween 20 at 45°C for 16 hours. After the hybridization, the GeneChip was washed and stained according to the protocol EukGE-WS2 attached to Affymetrix Fluidics Station. For the staining, streptavidin-phycoerythrin and biotinylated goat anti-streptavidin antibody were used. The stained GeneChip was scanned by using HP Argon Ion Laser Confocal Scanner (Hewlett Packard) to measure its fluorescence intensity. The fluorescence was measured at 570 nm by using an excitation light at 488 nm.

[0067] The quantitative data analyses were all conducted by using GeneChip Software (Affymetrix). To quantify RNA, an average of "differences [(Perfect match hybridization signal) - (Mismatch signal)]" was obtained for each probe family. When this value was 50 or higher, and quantitative values of RNA measured under two kinds of conditions diverge, preferably they diverge 1.8 times or more, it was determined that the gene expression had significantly "increased" or "decreased".

[0068] E7070, or ER-35748 or ER-68487 was added to a medium for HCT116-C9 at a concentration of 8 μ and culture was performed. The results of analysis of RNA extracted from the cells at 12 hours by using GeneChip were compared with the results of RNA analysis for HCT116-C9 cells obtained in the same manner except for not adding the drug. As a result, a list of genes of which expression levels were increased in common by the treatments with three types of drugs, E7070, ER-35748 and ER-68487 (GenBank registration numbers are also shown (the same shall apply in other tables shown below)) is shown in Table 1. Similarly, a list of genes of which expression levels were decreased in common by the treatments with the three types of drugs is shown in Table 2.

Table 1

Registration number	Gene name
D59253	NCBP interacting protein 1
D78514	Ubiquitin-conjugating enzyme
D83767	Rep-8
HG1139-HT4910	Fk506-Binding protein
HG3484-HT3678	Cdc-like kinase 1 (Clk1)
J04152	Tumor-associated antigen GA733-1, M1S1

Table 1 (continued)

Registration number	Gene name
M21154	S-adenosylmethionine decarboxylase
M60724	p70 Ribosomal S6 kinase alpha-1
M84349	Transmembrane protein CD59
S61953	c-ErbB3 receptor tyrosine kinase
U52960	RNA polymerase II complex component SRB7
U84720	Export protein RAE1
U92014	Defective mariner transposon Hsmar2
Z18951	Caveolin

Table 2

Registration number	Gene name
AB000409	Serine/threonine protein kinase MNK1
AB000450	Putative serine/threonine protein kinase VRK2
AB002380	Leukemia-associated Rho GEF
AB003102	Proteasome 26S subunit p44.5
AC002045	CIT987SK-A-589H1
AF002020	Niemann-Pick C disease protein (NPC1)
AF008445	Phospholipid scramblase
D00723	Hydrogen carrier protein, glycine synthase
D14659	KIAA0103
D21852	KIAA0029
D26535	Dihydrolipoamide succinyltransferase
D28364	Annexin II
D29677	KIAA0054
D29810	Unknown product
D29956	Ubiquitin specific protease 8
D30756	KIAA0049
D31883	Actin-binding LIM protein
D32002	Nuclear cap binding protein
D38521	KIAA0077
D38552	KIAA0073
D38553	KIAA0074
D43947	KIAA0100
D43948	ch-TOG
D50645	SDF2
D50663	Dynein (TCTEL1)
D50912	RNA-binding motif protein 10
D50916	Ubiquitination factor E4A
D61391	PAP39
D63480	KIAA0146
D63506	Syntaxin-binding protein 3
D63875	TPR-containing/SH2-binding protein
D63880	KIAA0159
D78586	Multifunctional protein CAD
D79983	KIAA0161
D79987	KIAA0165
D79988	KIAA0166
D79991	KIAA0169

Table 2 (continued)

Registration number	Gene name
D83776	KIAA0191
D83781	KIAA0197
D84307	Phosphoethanolamine cytidylyltransferase
D86981	Amyloid precursor protein-binding protein 2
D87435	KIAA0248
D87446	KIAA0257
D87448	DNA topoisomerase II-binding protein
D87743	Solute carrier family 9
HG1869-HT1904	Male enhanced antigen
HG2379-HT3996	Serine hydroxymethyltransferase, cytosolic
HG4094-HT4364	Transcription factor Lsf-Id
J04088	DNA topoisomerase II (top2)
J04501	Muscle glycogen synthase
J04543	Synexin
L06845	Cysteinyl-tRNA synthetase
L07033	Hydroxymethylglutaryl-CoA lyase
L07540	Replication factor C, 36-kDa subunit
L07597	Ribosomal protein S6 kinase 2
L07758	IEF SSP 9502
L21936	Succinate dehydrogenase flavoprotein subunit
L25444	TAFII70-alpha
L25931	Lamin B receptor
L33075	Ras GTPase-activating-like protein IQGAP1
L33881	Protein kinase C iota isoform
L38810	Proteasome 26S subunit p45
L40395	Eukaryotic initiation factor 2B-beta
L41870	Retinoblastoma susceptibility protein (RB1)
L47276	Alpha topoisomerase truncated-form
M15796	PCNA
M19267	Tropomyosin
M22632	Mitochondrial aspartate aminotransferase
M23379	GTPase-activating protein ras p21
M24486	Prolyl 4-hydroxylase alpha subunit
M29204	DNA-binding factor
M29550	Calcineurin A1
M30496	Ubiquitin carboxyl-terminal hydrolase
M33518	HLA-B-associated transcript 2 (BAT2)
M34309	Epidermal growth factor receptor HER3
M37400	Cytosolic aspartate aminotransferase
M55905	Mitochondrial NAD(P) + dependent malic enzyme
M58525	Catechol-O-methyltransferase
M59911	Integrin alpha-3
M61764	Gamma-tubulin
M62994	Filamin B
M74089	TB1
M85085	Cleavage stimulation factor
M86707	Myristoyl CoA: protein N-myristoyl transferase
M87338	Replication factor C, 40-kDa subunit (A1)
M87339	Replication factor C, 37-kDa subunit (RFC4)
M88163	Global transcription activator (hSNF2/SWI2)

Table 2 (continued)

Registration number	Gene name
M91432	Medium-chain acyl-CoA dehydrogenase (MCAD)
M92439	Leucine-rich protein
M93056	Monocyte/neutrophil elastase inhibitor
M95809	Basic transcription factor 62kD subunit (BTF2)
M95929	Homeobox protein PHOX1
M97935	Transcription factor ISGF-3
S58544	75kDa infertility-related sperm protein
S59184	RYK = related to receptor tyrosine kinase
S72904	APK1 antigen = MAb KI recognized
S78085	PDCD2, Rp8 homolog
S80343	Arginyl-tRNA synthetase (ArgRS)
U01062	Inositol 1,4,5-triphosphate receptor type 3
U02566	Receptor tyrosine kinase tif
U04285	Lysosomal acid lipase
U06631	H326
U07231	G-rich sequence factor-1
U07681	Isocitrate dehydrogenase 3 (NAD+) alpha
U079I9	Aldehyde dehydrogenase 6 (ALDH6)
U08815	Splicesomal protein SAP61
U10324	Nuclear factor NF90
U11791	Cyclin H
U15174	Nip3
U15306	DNA-binding protein NFX1
U18291	CDC16HS
U18934	Receptor tyrosine kinase DTK
U20979	Chromatin assembly factor-I p150 subunit
U22233	Methylthioadenosine phosphorylase
U23028	Eukaryotic initiation factor 2B-epsilon
U23946	LUCA15
U26648	Syntaxin 5A
U27459	Origin recognition complex protein 2 homolog
U27460	Uridine diphosphoglucose pyrophosphorylase
U28413	Cockayne syndrome complementation group A
U28811	Cystein-rich fibroblast growth factor receptor
U28831	Immuno-reactive with anti-PTH Ab
U28963	Gps2
U30313	Diadenosine tetraphosphatase
U30521	P311 HUM -3.1
U30827	Splicing factor SRp40-3 (SRp40)
U30828	Splicing factor SRp55-2 (SRp55)
U34252	Gamma-aminobutyraldehyde dehydrogenase
U34683	Glutathione synthetase
U36341	SLC6A8
U40282	Integrin-linked kinase
U46006	Smooth muscle LIM protein (h-SmLIM)
U49844	FRAP-related protein (FRP1/ATR)
U50078	Guanine nucleotide exchange factor p532
U50939	Amyloid precursor protein-binding protein 1
U53468	NADH: ubiquinone oxidoreductase subunit B13
U57629	Retinitis pigmentosa GTPase regulator

Table 2 (continued)

Registration number	Gene name
U60808	CDP-diacylglycerol synthase
U61145	Enhancer of zeste homolog 2
U61263	Acetolactate synthase homolog
U63743	Mitotic centromere-associated kinesin (MCAK)
U65785	Oxygen-regulated protein ORP150
U72514	C2f
U72515	C3f
U76764	CD97
U77413	O-linked GlcNAc transferase
U77949	Cdc6-related protein (HsCDC6)
U79241	Clone 23759
U80034	Mitochondrial intermediate peptidase precursor
U81554	CaM kinase II isoform
U85611	DNA-PK interaction protein (KIP)
U89606	Pyridoxal kinase
U90426	Nuclear RNA helicase
U94319	Transcription coactivator p75 (DFS70)
U95740	362G6.1
U97188	Putative RNA binding protein KOC
X06745	DNA polymerase alpha subunit
X13482	U2 snRNP-specific A protein
X51956	Neuron specific (gamma) enolase
X53587	Integrin beta-4
X54199	GARS-AIRS-GART
X54867	NKG2-A
X59871	T cell factor 1
X61100	75 kDa subunit NADH dehydrogenase precursor
X66364	Serine/threonine protein kinase PSSALRE
X68836	S-adenosylmethionine synthetase
X70476	Subunit of coatomer complex
X75535	PxF
X81003	HCG V
X84740	DNA ligase III
X94754	Yeast methionyl-tRNA synthetase homologue
X98248	Sortilin
X99209	Arginine methyltransferase
Y08612	Nup88
Y08682	Carnitine palmitoyltransferase I type I
Y13115	Serine/threonine protein kinase SAK
Z11518	Histidyl-tRNA synthetase
Z17227	Transmenbrane receptor protein CRF2-4
Z46629	SOX9
Z68747	Imogen 38

[0069] Two kinds of E7070-resistant strains (C9-C1 and another resistant strain (C9-C4) obtained in the same method as C9-C1) were treated with 8 μM of E7070 for 12 hours in the same manner as described above, and variations of the gene expression were analyzed by using GeneChip for the genes listed in Tables 1 and 2. As a result, no gene that increased or decreased 1.8 times or more was observed in the both strains.

[0070] From the above results, it is apparent that genes of which expression levels are changed in common by the treatments with these three types of anticancer agents can be used as surrogate markers of antitumor effect of E7070

and its related compounds by measuring each of their changes or changes of two or more of them in combination.

Example 3 Gene expression analysis by quantitative PCR

[0071] In order to confirm that variations in gene expression obtained in Example 2 reflected the susceptibility of a tumor cell to E7070, changes of gene expression in E7070-susceptible cells and E7070-resistant cells were examined by quantitative PCR using RNA shown in Example 1, SYBR Green and ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems).

[0072] The operation was performed in two steps of a reverse transcription reaction and PCR reaction. The reverse transcription reaction as the first step was carried out by adding 1 x TaqMan RT buffer, 5.5 mM $MgCl_2$, 500 μM dNTP mix, 2.5 μM oligo $d(T)_{16}$ primer, 0.4 U/μl of RNase inhibitor and 1.25 U/μl of Multiscribe Reverse Transcriptase (Perkin-Elmer Applied Biosystems) to 1 μg of the total RNA, keeping the mixture at 25°C for 10 minutes and then heating it at 48°C for 30 minutes. The reaction was terminated by heating at 95°C for 5 minutes.

[0073] The obtained cDNA was subjected to the PCR reaction as the second step. The PCR reaction was carried out in a reaction system composed of 4 ng of cDNA, 1 x SYBR PCR buffer, 3 mM $MgCl_2$, 200 μM each of dATP, dCTP and dGTP, 400 μMdUTP, 200 nM primer pair, 0.01 U/μl AmpErase UNG and 0.025 U/μl AmpliTaq Gold DNA Polymerase (Perkin-Elmer Applied Biosystems). The PCR reaction was performed with reactions at 50°C for 2 minutes and at 95°C for 10 minutes followed by a cycle of reactions at 95°C for 20 seconds, at 55°C for 20 seconds and at 72°C for 30 seconds, which are repeated 40 times. As the primers, oligonucleotides having the nucleotide sequences of SEQ ID NOS: 1 and 2 were used for GAPDH, those of SEQ ID NOS: 3 and 4 for S80343, those of SEQ ID NOS: 5 and 6 for U07919, those of SEQ ID NOS: 7 and 8 for U11791, those of SEQ ID NOS: 9 and 10 for M95809, those of SEQ ID NOS: 11 and 12 for U18291, those of SEQ ID NOS: 13 and 14 for U63743, and those of SEQ ID NOS: 15 and 16 for M61764.

[0074] The mRNA level in each specimen was quantified by measuring fluorescence intensity. In comparison of two or more specimens, quantitative values were corrected based on the mRNA level of GAPDH in each specimen. The relationships between the drug treatment time and each mRNA level are shown in Figs. 2 to 5, where the quantified value at 0 hour after the drug treatment was used as a control (100%). The genes showed the largest expression variation in HCT116-C9 with the highest susceptibility (Fig. 2), and in the susceptible strain LX-1, all the genes except for S80343 showed expression variations although the variation magnitudes were smaller than those in HCT116-C9 (Fig. 3). On the other hand, no expression variation was observed for the resistant strain HCT116-C9-C1 (Fig. 4), and in the resistant strain LX-1-E2, which showed some growth inhibitory effect at the drug concentration of 8 μ used in the experiment, U11791 showed an expression change and M95809 and U18291 showed a slight expression change (Fig. 5).

[0075] The variations in expression of the gene retrieved in Example 2 correlated to the susceptibility to E7070, and it was confirmed that the genes retrieved in Example 2 could be used solely or in combination as surrogate markers of antitumor effect of E7070 and its related compounds.

Example 4 Analysis by ELISA

[0076] Among the genes listed in Table 2 in Example 2, the expression level of X51956 (Human ENO2 gene for neuron specific (gamma) enolase), which has been reported to be secreted out of the cell, was examined by the already reported ELISA method (Duncan M. E., McAleese S. M., Booth N. A., Melvin W. T., and Fothergill J. E., J. of Immunol. Methods, 151, 227-236, 1992, Yamaguchi K., Aoyagi K., Urakami K., Fukutani T., Maki N., Yamamoto S., Otsubo K., Miyake Y., and Kodama T., Jpn. J. Cancer Res., 86, 698-705, 1995). The actual measurement was carried out by using NSE ELISA Kit produced by Eiken Chemical (Tokyo) according to the attached reference.

Example 5 Gene expression analysis in three kinds cancers

[0077] Gene expression changes in three kinds of cancers of HCT116-C9 (human colon cancer cell strain), MDA-MB-435 (human breast cancer cell strain) and MOLT-4 (human T lymphoblastic leukemia cell strain) caused by E7070 (treated with 8 μM for 12 hours) were examined by using GeneChip in the same manner as in Example 2.

[0078] A list of the genes of which expression was enhanced 1.8 times or more in common in the three kinds of cancers is shown in Table 3. Further, a list of the genes of which expression was inhibited 1.8 times or more in common in the three kinds of cancers is shown in Table 4. Since the genes of which expression varies (enhanced or reduced) in common in these strains of the three kinds of cancers established from cancer patients with totally different genetic backgrounds are very likely to be more closely associated with the E7070 antitumor action mechanism common to the tumor cells, it is considered that they can be used as valid markers when susceptibility of a tumor cell to E7070 and its related compounds is tested.

Table 3

Registration number	Gene name
D78514	Ubiquitin-conjugating enzyme
D83767	Rep-8
HG1139-HT4910	Fk506-Binding protein
HG3484-HT3678	Cdc-like kinase 1 (Clk1)
M21154	S-adenosylmethionine decarboxylase
M60724	p70 Ribosomal S6 kinase alpha-1
S61953	c-ErbB3 receptor tyrosine kinase
U52960	RNA polymerase II complex component SRB7
U84720	Export protein RAE1
U92014	Defective mariner transposon Hsmar2

Table 4

Registration number	Gene name
AB000409	Serine/threonine protein kinase MNK1
AB000450	Putative serine/threonine protein kinase VRK2
AC002045	CIT987SK-A-589H1
AF008445	Phospholipid scramblase
D00723	Hydrogen carrier protein, glycine synthase
D14659	KIAA0103
D21852	KIAA0029
D26535	Dihydrolipoamide succinyltransferase
D32002	Nuclear cap binding protein
D38521	KIAA0077
D38553	KIAA0074
D43947	KIAA0100
D50912	RNA-binding motif protein 10
D61391	PAP39
D63480	KIAA0146
D63880	KIAA0159
D78586	Multifunctional protein CAD
D79983	KIAA0161
D79991	KIAA0169
D83781	KIAA0197
D84307	Phosphoethanolamine cytidylyltransferase
D87446	KIAA0257
HG1869-HT1904	Male enhanced antigen
HG2379-HT3996	Serine hydroxymethyltransferase, cytosolic
HG4094-HT4364	Transcription factor Lsf-Id
J04088	DNA topoisomerase II (top2)
L07758	IEF SSP 9502
L21936	Succinate dehydrogenase flavoprotein subunit
L25931	Lamin B receptor
L38810	Proteasome 26S subunit p45
L41870	Retinoblastoma susceptibility protein (RB1)
L47276	Alpha topoisomerase truncated-form
M19267	Tropomyosin
M22632	Mitochondrial aspartate aminotransferase

Table 4 (continued)

Registration number	Gene name
M29204	DNA-binding factor
M29550	Calcineurin A1
M30496	Ubiquitin carboxyl-terminal hydrolase
M34309	Epidermal growth factor receptor HER3
M55905	Mitochondrial NAD(P) + dependent malic enzyme
M61764	Gamma-tubulin
M85085	Cleavage stimulation factor
M87338	Replication factor C, 40-kDa subunit (A1)
M91432	Medium-chain acyl-CoA dehydrogenase (MCAD)
M92439	Leucine-rich protein
M93056	Monocyte/neutrophil elastase inhibitor
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M97935	Transcription factor ISGF-3
S72904	APK1 antigen = MAb KI recognized
S78085	PDCD2, Rp8 homolog
U01062	Inositol 1,4,5-triphosphate receptor type 3
U07231	G-rich sequence factor-1
U08815	Splicesomal protein SAP61
U11791	Cyclin H
U15306	DNA-binding protein NFX1
U18291	CDC16HS
U18934	Receptor tyrosine kinase DTK
U20979	Chromatin assembly factor-I p150 subunit
U22233	Methylthioadenosine phosphorylase
U23028	Eukaryotic initiation factor 2B-epsilon
U23946	LUCA15
U28831	Immuno-reactive with anti-PTH Ab
U28963	Gps2
U30828	Splicing factor SRp55-2 (SRp55)
U34683	Glutathione synthetase
U40282	Integrin-linked kinase
U49844	FRAP-related protein (FRP1/ATR)
U50939	Amyloid precursor protein-binding protein 1
U57629	Retinitis pigmentosa GTPase regulator
U61145	Enhancer of zeste homolog 2
U72514	C2f
U77413	O-linked GlcNAc transferase
U77949	Cdc6-related protein (HsCDC6)
U79241	Clone 23759
U80034	Mitochondrial intermediate peptidase precursor
U81554	CaM kinase II isoform
U89606	Pyridoxal kinase
U94319	Transcription coactivator p75 (DFS70)
X06745	DNA polymerase alpha subunit
X13482	U2 snRNP-specific A protein
X51956	Neuron specific (gamma) enolase
X54199	GARS-AIRS-GART
X61100	75 kDa subunit NADH dehydrogenase precursor
X68836	S-adenosylmethionine synthetase
X70476	Subunit of coatomer complex

Table 4 (continued)

Registration number	Gene name
X75535	PxF
X99209	Arginine methyltransferase
Y08612	Nup88
Y08682	Carnitine palmitoyltransferase I type I
Z17227	Transmembrane receptor protein CRF2-4

Industrial Applicability

[0079] According to the present invention, by measuring an expression level or levels of a gene or genes listed in Tables 3 and 4 in a tumor cell collected from a cancer patient treated with an anticancer agent represented by the general formula (I) or causing the anticancer agent represented by the general formula (I) to act on a tumor cell collected from a cancer patient and measuring an expression level or levels of a gene or genes listed in Tables 3 and 4, susceptibility of the tumor cell to the anticancer agent can be examined.

Sequence Listing

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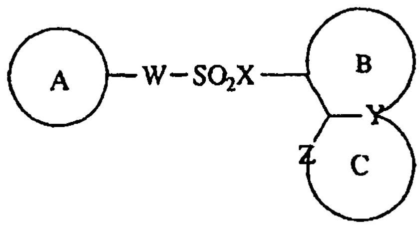
Claims

1. A method for testing susceptibility of a tumor cell to an anticancer agent, which comprises:

    1) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in a tumor cell collected from a cancer patient treated with an anticancer agent represented by the following general formula (I) :

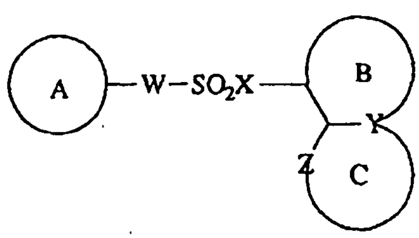
    

    (I)

    wherein A represents a monocyclic or bicyclic aromatic ring, which may be substituted,
    B represents a 6-membered unsaturated hydrocarbon ring or a 6-membered unsaturated heterocyclic ring containing one nitrogen atom as a heteroatom, each of which may be substituted,
    C represents a 5-membered heterocyclic ring containing one or two nitrogen atoms, which may be substituted,
    W represents a single bond or -CH=CH-,
    X represents -N(R^1)- or an oxygen atom,
    Y represents a carbon or nitrogen atom,
    Z represents -N(R^2)- or a nitrogen atom, and
    R^1 and R^2 may be identical or different and each represent a hydrogen atom or a lower alkyl group, and
    2) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in a tumor cell collected from the cancer patient before the treatment with the anticancer agent.

2. A method for testing susceptibility of a tumor cell to an anticancer agent, which comprises:

    1) causing an anticancer agent to act on a tumor cell collected from a cancer patient, said anticancer agent being represented by the following general formula (I):

    (I)

    wherein A represents a monocyclic or bicyclic aromatic ring, which may be substituted,
    B represents a 6-membered unsaturated hydrocarbon ring or a 6-membered unsaturated heterocyclic ring containing one nitrogen atom as a heteroatom, each of which may be substituted,
    C represents a 5-membered heterocyclic ring containing one or two nitrogen atoms, which may be substituted,
    W represents a single bond or -CH=CH-,
    X represents -N(R^1)- or an oxygen atom,
    Y represents a carbon or nitrogen atom,
    Z represents -N(R^2)- or a nitrogen atom, and
    R^1 and R^2 may be identical or different and each represent a hydrogen atom or a lower alkyl group,

2) measuring an expression level or levels of a gene or genes selected from the group consisting of the genes listed in Tables 3 and 4 in the tumor cell, and
3) determining that the tumor cell is susceptible to the anticancer agent when the expression level or levels of the gene or genes listed in Table 3 increase, or the expression level or levels of the gene or genes listed in Table 4 decrease in comparison with those in an untreated tumor cell.

3. The method according to claim 1 or 2, wherein the expression level or levels of gene or genes are measured by quantifying an RNA or RNAs which are a transcription product or products of the gene or genes by using a DNA microarray.

4. The method according to claim 1 or 2, wherein the expression level or levels of the gene or genes are measured by quantifying an RNA or RNAs which are a transcription product or products of the gene or genes by quantitative PCR.

5. A reagent for quantifying an RNA for use in the method as defined in claim 4, which comprises an oligonucleotide complementary to the RNA as a component.

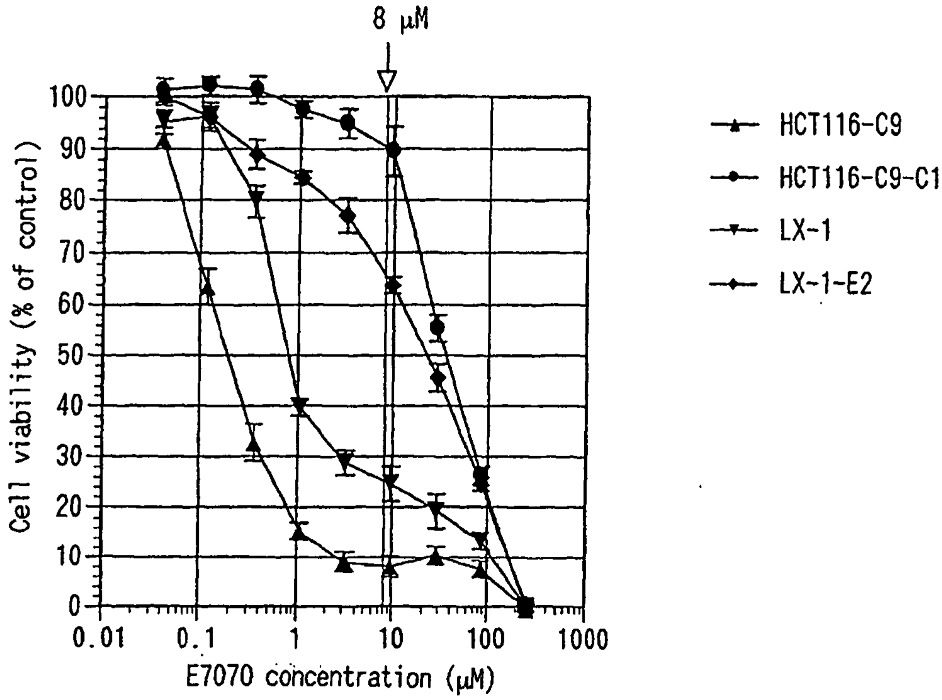
6. The method according to claim 1 or 2, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product or products of the gene or genes by an immunochemical method.

7. The method according to claim 6, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product or products of the gene or genes by ELISA.

8. The method according to claim 6, wherein the expression level or levels of the gene or genes are measured by quantifying a protein or proteins which are a gene product or products of the gene or genes by Western blotting.

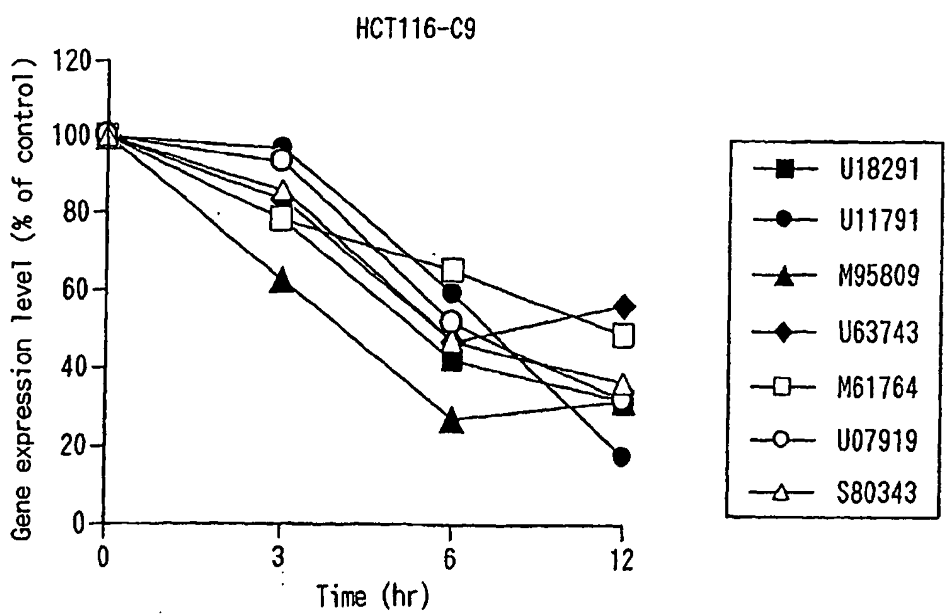
9. An immunoassay reagent used in the method as defined in claim 6, which comprises an antibody directed to the protein as a component.

10. The method according to claim 1 or 2, wherein A represents benzene or pyridine which may be substituted, B represents benzene which may be substituted, C represents pyrrole which may be substituted, W represents a single bond, and X and Z both represent -NH-.



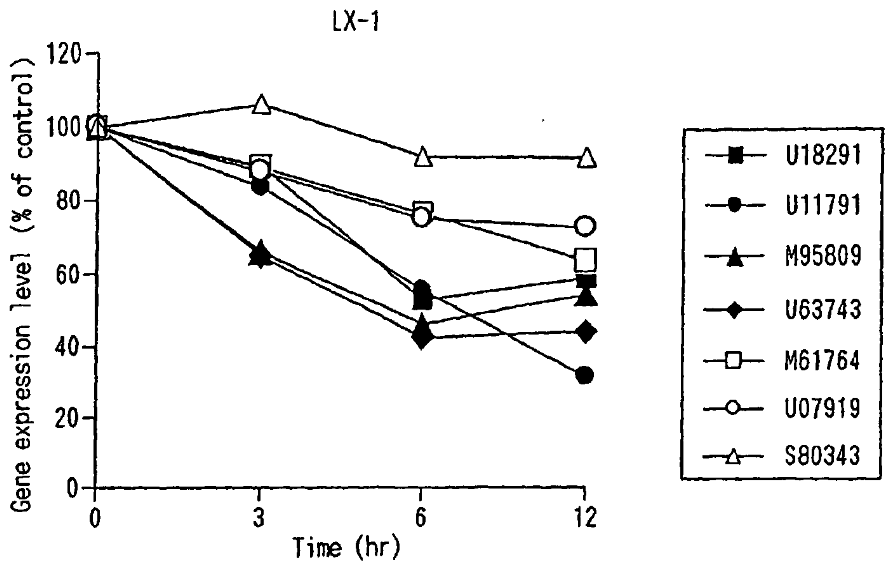
8 μM
100
90
80
70
60
50
40
30
20
10
0
Cell viability (% of control)
0.01
0.1
1
10
100
1000
E7070 concentration (μM)
HCT116-C9
HCT116-C9-C1
LX-1
LX-1-E2

Fig. 1



HCT116-C9
120
100
80
60
40
20
0
Gene expression level (% of control)
0
3
6
12
Time (hr)
U18291
U11791
M95809
U63743
M61764
U07919
S80343

Fig. 2


LX-1
Gene expression level (% of control)
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100
80
60
40
20
0
0
3
6
12
Time (hr)
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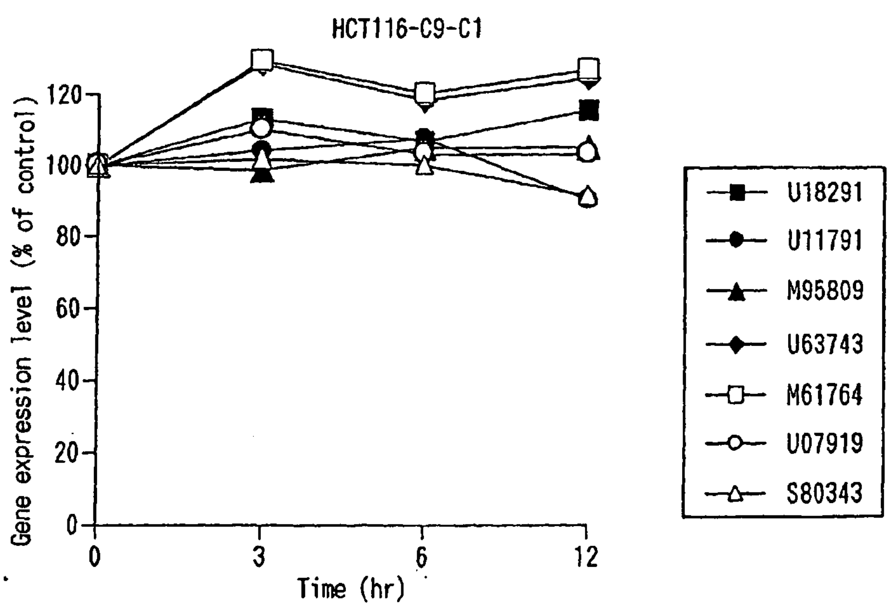
U07919
S80343

Fig. 3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Time (hr)
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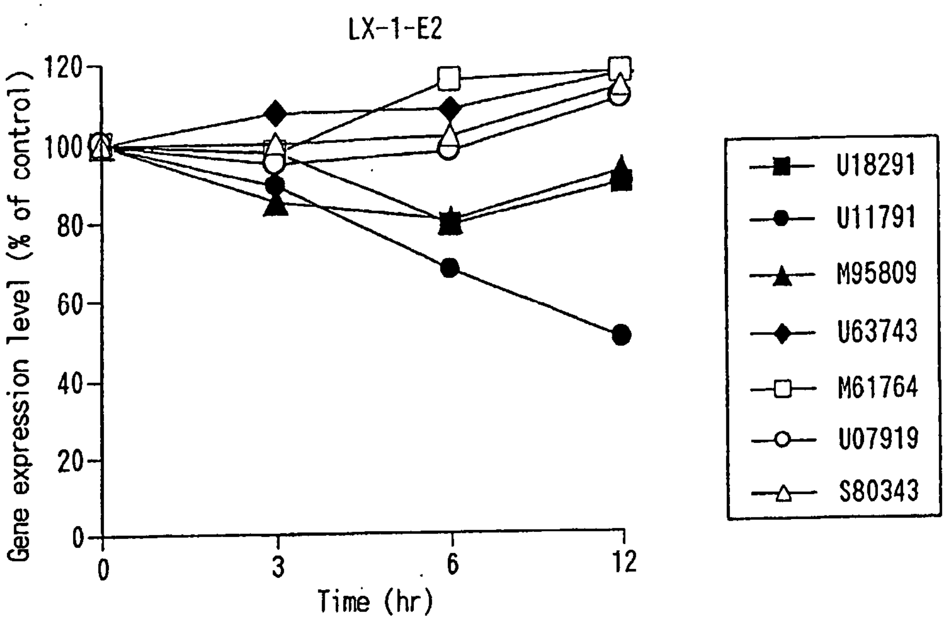
S80343

Fig. 4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Gene expression level (% of control)
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60
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20
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0
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6
12
Time (hr)
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U11791
M95809
U63743
M61764
U07919
S80343

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/10282

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl^7 C12Q 1/68, C12N 15/09, C12M 1/00, G01N 33/53, G01N 33/566, G01N 37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl^7 C12Q 1/68, C12N 15/09, C12M 1/00, G01N 33/53, G01N 33/566, G01N 37/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
REGISTRY (STN), CA (STN), MEDLINE (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95/07276 A1 (Eisai Co., Ltd.), 16 March, 1995 (16.03.1995), & JP 7-165708 A & EP 673937 A1 & US 5721246 A & US 5767283 A & AU 9476237 A & NO 9501813 A & FI 9502272 A & CN 1114506 A & NZ 273073 A & HU 71551 T & RU 2121997 C1 & KR 174752 B1	1-10
A	OWA, T. et al., "Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle", J. Med. Chem., 23 September, 1999 (23.09.1999), Vol.42, No.19, pages 3789 to 3799	1-10
A	OWA, T. et al., "A focused compound library of novel N-(7-indolyl)benzenesulfonamides for the discovery of potent cell cycle inhibitors", Bioorg. Med. Chem. Lett., 05 June, 2000 (05.06.2000), Vol.10, No.11, pages 1223 to 1226	1-10

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 February, 2002 (08.02.02)	19 February, 2002 (19.02.02)

Name and mailing address of the ISA/	Authorized officer
Japanese Patent Office	
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No. PCT/JP01/10282

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	OZAWA, Y. et al., "E7070, a novel sulphonamide agent with potent antitumour activity in vitro and in vivo", Eur. J. Cancer, November, 2001, Vol.37, No.17, pages 2275 to 2282	1-10
A	KUDOH, K. et al., "Monitoring the expression profiles of doxorubicin-induced and doxorubicin-resistant cancer cells by cDNA microarray", Cancer Res., 01 August, 2000 (01.08.2000), Vol.60, No.15, pages 4161 to 4166	1-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)